PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP2004/012743	10.11.2004	10.11.2003

International Patent Classification (IPC) or both national classification and IPC
C12N15/82, A01H5/00

Applicant
ICON GENETICS AG

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☒ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☒ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☒ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

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WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/EP2004/012743

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing:

   ☐ contained in the international application as filed.

   ☐ filed together with the international application in computer readable form.

   ☒ furnished subsequently to this Authority for the purposes of search.

3. ☒ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

Box No. II Priority

1. ☒ The validity of the priority claim has not been considered because the International Searching Authority does not have in its possession a copy of the earlier application whose priority has been claimed or, where required, a translation of that earlier application. This opinion has nevertheless been established on the assumption that the relevant date (Rules 43*bis*.1 and 64.1) is the claimed priority date.

2. ☐ This opinion has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rules 43*bis*.1 and 64.1). Thus for the purposes of this opinion, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 56

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination *(specify)*:

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for the whole application or for said claims Nos. 56

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form ☐ has not been furnished

☐ does not comply with the standard

the computer readable form ☐ has not been furnished

☐ does not comply with the standard

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See separate sheet for further details

Box No. IV Lack of unity of invention

1. ☒ In response to the invitation (Form PCT/ISA/206) to pay additional fees, the applicant has:

   ☐ paid additional fees.

   ☐ paid additional fees under protest.

   ☒ not paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is

   ☐ complied with

   ☒ not complied with for the following reasons:

   see separate sheet

4. Consequently, this report has been established in respect of the following parts of the international application:

   ☐ all parts.

   ☒ the parts relating to claims Nos. 1-55 and 57

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-55, 57
	No:	Claims	
Inventive step (IS)	Yes:	Claims	7-8, 28, 44-45, 55
	No:	Claims	1-6, 9-27, 29-43, 46-54, 57
Industrial applicability (IA)	Yes:	Claims	1-55, 57
	No:	Claims	

2. Citations and explanations

   see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Re Item IV

Lack of unity of invention

The ISA considers that the present application lacks unity and identifies the following groups of inventions in the international application :

A. Claims 1-55 and 57 (completely) :

A stably transformed plant containing in cell nuclei a heterologous DNA having s sequence encoding a RNA replicon operably linked or linkable to a transcription promoter, the sequence encoding a RNA replicon functions exhibiting at selected localities function-conservative differences causing an increased frequency of replicon formation compared to a RNA replicon not exhibiting said differences.
A process of expressing a sequence of interest in a plant using such a modified RNA replicon.
A nucleic acid encoding a RNA replicon wherein the sequences for replicon function exhibit at selected localities function-conservative differences being capable of causing an increased frequency of replicon formation compared to a RNA replicon not exhibiting said differences.

B. Claim 56 (completely).

A process of expressing a sequence of interest in a plant comprising transforming a plant with a suspension of Agrobacteria containing in the T-DNA a heterologous DNA encoding a replicon operably linked or linkable to a transcription factor whereby the suspension of Agrobacteria has a concentration of cells of said Agrobacteria corresponding to a calculated optical density at 600 nm of at most 0.04.

The common concept linking these two groups of inventions can be seen as the provision of a system for expressing a sequence of interest in a plant comprising transforming said plant with a sequence encoding a RNA replicon comprising the sequence of interest.

The documents D1 and D2 disclose such systems. Therefore, the common concept linking the two groups of invention mentioned above cannot be considered as novel (article 33(2)

PCT) or inventive (article 33(3) PCT) and the present application lacks unity in the sense of Rule 13.1 PCT.

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. The present application refers to stably transformed plant, plant parts or plant cells containing in the cell nuclei a heterologous DNA having a sequence encoding a RNA replicon operably linked or linkable to a transcription promoter whereby the sequences for replicon function of said RNA replicon exhibit at selected localities function-conservative differences causing an increased frequency of replicon formation compared to a RNA replicon not exhibiting said differences. The application also refers to processes of expressing a sequence of interest in a plant comprising the use of such a modified RNA replicon and a nucleic acid sequence of such a modified RNA replicon.

2. Reference is made to the following documents :

   D1: US-B1-6 632 980 (YADAV NARENDRA S ET AL) 14 October 2003 (2003-10-14)

   D2: MALLORY A C ET AL: "THE AMPLICON-PLUS SYSTEM FOR HIGH-LEVEL EXPRESSION OF TRANSGENES IN PLANTS" NATURE BIOTECHNOLOGY, NATURE PUBLISHING, US, vol. 20, no. 6, June 2002 (2002-06), pages 622-625.

   D3: SIMPSON C G ET AL: "Expression of a heterologous gene can be improved by mutation of cryptic splice sites" JOURNAL OF EXPERIMENTAL BOTANY, vol. 46, no. SUPPL., 1995, page 38 & ANNUAL MEETING OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY; ST. ANDREWS, SCOTLAND, UK; APRIL 3-7, 1995.

   D4: HASELOFF J ET AL: "REMOVAL OF A CRYPTIC INTRON AND SUBCELLULAR LOCALIZATION OF GREEN FLUORESCENT PROTEIN ARE REQUIRED TO MARK TRANSGENIC ARABIDOPSIS PLANTS BRIGHTLY" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA,

NATIONAL ACADEMY OF SCIENCE. WASHINGTON, US, vol. 94, March 1997 (1997-03), pages 2122-2127.

D5: ROSE A B: "Requirements for intron-mediated enhancement of gene expression in Arabidopsis" RNA 01 NOV 2002 UNITED STATES, vol. 8, no. 11, 1 November 2002 (2002-11-01), pages 1444-1453.

D6: KOZIEL M G ET AL: "OPTIMIZING EXPRESSION OF TRANSGENES WITH EMPHASIS ON POST-TRANSCRIPTIONAL EVENTS" PLANT MOLECULAR BIOLOGY, NIJHOFF PUBLISHERS, DORDRECHT, NL, vol. 32, 1996, pages 393-405.

D7: SIMPSON C G ET AL: "Efficient splicing of an AU-rich antisense intron sequence." PLANT MOLECULAR BIOLOGY. JAN 1993, vol. 21, no. 2, January 1993 (1993-01), pages 205-211.

3. Lack of inventive step; article 33(3) PCT.

The expression of protein of interest using RNA replicons was already known in the prior art.
D1 discloses a binary viral expression system where one component is an inactive replicon containing cis-acting viral sequences required for replication and unable to replicate episomally and the second component is a chimeric transactivating gene comprising a regulated promoter operably linked to the coding region for a protein that can transactivate replicon replication. D1 foresees the use of single stranded RAN viruses.
D2 discloses the amplicon-plus system for high-level expression of transgene in plants. This system is based on the use of transgenic plant lines that encode a replicating potatovirus X vector carrying a gene of interest.

In the light of this prior art, the problem to be solved by the present application can be seen as the provision of an improved system of protein expression based on a RNA virus replicon.

The present application solves this problem by the provision of RNA replicons exhibiting at selected localities function-conservative differences causing an increased frequency of replicon formation. In more details, the function conservative

differences are (1) the elimination of A/U rich regions, (2) the elimination of cryptic splicing sites and (3) the introduction of introns near or within A/U rich regions.

The problems due to cryptic splicing sites in heterologous genes to be expressed in plants were already known in the prior art. The suppression of cryptic splicing sites has been shown to enhance the expression of the transgenes (see documents D3 and D4). Therefore, the International Search Authority (ISA) considers that the removal of cryptic splicing sites near A/U rich regions in the RNA replicon - which is a heterologous DNA - in order to increase the frequency of replicon formation cannot be considered as inventive in the sense of article 33(3) PCT.

The use of introns in a coding sequence or in the 5' untranslated region of a mRNA to enhance gene expression was also known, especially in monocot but also in dicot species as illustrated in D5 and D6. However, it has never been suggested that such sequence should be positioned in the vicinity or within an A/U rich region. Therefore, the mere addition of an intron in the 5' untranslated region of an mRNA or in the coding sequence of a heterologous DNA in order to enhance gene expression cannot be considered as inventive.

D7 discloses the splicing of non-intron AU rich regions and suggests that cryptic splicing of AU-rich sequences may have implications for the successful expression of AU-rich heterologous genes in transgenic plants (p. 210, right-hand column, lines 15-25). In the light of D7, the skilled person would have needed no inventive activity to consider removing AU-rich regions from heterologous genes.

The ISA is of the opinion that the use of a RNA replicon composed from several segment which can be rearranged by site-specific recombination cannot be considered as inventive (the reconstruction of functional transgenes by trans-splicing or recombinase-mediated rearrangement was already known in the art). Also the use of agroinfiltration to introduce a transgene in a plant was well known and cannot be considered as inventive.

Therefore, the subject-matter of claims 1-6, 9-27, 29-43, 46-54 and 57 cannot be considered as inventive (article 33(3) PCT).

Re Item VIII

Certain observations on the international application

1. Claim 1 lacks clarity for the following reasons :
   (i) Claim 1 refers, inter alia, to a stably transformed plant cell culture. It is not clear if all cells of said culture have to be transformed what renders the scope of the claim unclear.
   (ii) The "selected localities" referred to in claim 1 are not characterize by any technical feature what renders the claim unclear.
   (iii) It is not clear what should be the "function-conservative difference" referred to in claim 1. Said "function-conservative differences" are only characterized by the result to be achieved by said differences. According to the PCT International Search and Examination Guidelines as in force from March 25, 2004, chapter 5.35 : "The area defined by the claims must be as precise as the invention allows. As a general rule, claims which attempt to define the invention, or a feature thereof, by a result to be achieved should be objected to as lacking clarity".
   This remark also applies to claim 2.
   (iv) It is not clear what should be considered as an "increased frequency of replicon formation" what renders the scope of the claim unclear.

   These remarks also apply to claim 57.

2. Claim 2 refers to a "high A/U content" in a transcript. It is not clear what should be considered as a high A/U content what renders the scope of the claim unclear.
   This remark also applies mutatis mutandis to claims 3 (localities of high A/U content), 4-6, 7 (A/U-rich localities), 13, 39-44, 48.

3. In claim 14, the term "preferably" is used. The attention of the applicant is drawn to the fact that, according to the PCT International Search and Examination Guidelines as in force from March 25, 2004, chapter 5.40 : Expressions, like "preferably", "for example", "such as" or "more particularly" should be regarded "as having no limiting

effect on the scope of a claim; that is to say, the feature following any such expression should be regarded as entirely optional".
This remark also applies to claims 50 and 55.

4. Claim 15 refers to function-conservative differences which should be "function-silent". The ISA fails to understand what should "function-silent" differences precisely be.

5. Claim 49 c) refers to the step of identifying in the sequence of the product of a RT-PCR a selected locality as a locality of an undesired splicing event. However, there is not any indication as how to achieve this.